NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES (U.S.)

ANNUAL REPORTS

INTRAMURAL RESEARCH PROGRAMS

OCTOBER 1, 1985 TO SEPTEMBER 30, 1986

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PREFACE

The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is the lead Institute for a research program with a formidable goal; to elucidate the nature, cause and progression of a wide spectrum of diseases which heretofore largely defied the probings of biomedical science. Disorders such as arthritis, diseases of skeletal support structures, and diseases of the skin, while not usually fatal, are among the most common causes of pain, disability, and disfigurement. In addition to the toll they exact in terms of human suffering, the economic impact of these disorders ranges into billions of dollars each year for medical care and lost productivity.

Understanding such complex disorders so that they may eventually be controlled, effectively treated, and prevented is the goal of all studies conducted and supported by NIAMS. It is an understanding that must begin at the cellular level, in basic research studies, and be extended by clinical investigations and, ultimately, where needed, by developmental research.

The intramural research program of NIAMS covers a wide range of these investigations. Administratively, it is a relatively young entity created when the NIAMS became a new Institute within the framework of NIH. Its achievements, productivity and traditions, however, have a long and positive history. Our intramural research staff is generally known as a highly productive and innovative group of scientists. This annual report represents a chronicle of the research efforts of the year just finished and describes the opportunities and plans for further research to come. The achievements described here reflect the development of new and promising knowledge which would not be possible without the original ideas and dedicated efforts of the talented investigators who participate in our fight against arthritis, musculoskeletal and skin diseases, and the credit for these advances belongs to them.

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NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

ACTIVE PROJECTS

- Z01 AR 27000-24 LPB
- Z01 AR 27001-12 LPB
- Z01 AR 27012-02 LPB
- Z01 AR 41020-19 ARB
- Z01 AR 41021-18 ARB
- Z01 AR 41022-14 ARB
- Z01 AR 41023-12 ARB
- Z01 AR 41025-15 ARB
- Z01 AR 41040-14 ARB
- Z01 AR 41048-07 ARB
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- Z01 AR 41071-02 ARB
- Z01 AR 41072-02 ARB
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INACTIVE PROJECTS

- Z01 AM 27002-23 LPB
- Z01 AM 27004-16 LPB

TERMINATED PROJECTS

- Z01 AM 27003-17 LPB
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- Z01 AM 27008-27 LPB
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ANNUAL REPORT OF THE LABORATORY OF PHYSICAL BIOLOGY
NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

Muscle Contraction

This year evidence was found that the molecular process that drives the contractile mechanism in muscle cells may be localized in the "hinge region" of the myosin molecule. Considerable progress was also made in our understanding of the structures that position the myosin-containing filaments in the middle of the sarcomere, and in the relation between ligand binding to acto-myosin crossbridges in rigor muscle fibers and to acto-myosin subfragment 1 in solution. The influence of the regulatory protein, tropomyosin, on the formation of the newly discovered "low ionic strength" crossbridge was examined by two-dimensional X-ray diffraction, using a novel X-ray detector.

Force generating mechanisms in muscle fibers.

The molecular processes underlying force generation in muscle cells are not known. A widely held idea (Huxley, 1969) is that the "head" of the myosin molecule (subfragment 1 moiety, S1) can bind to the actin-containing filament in different orientations and that force is developed by a transition between these orientations. Evidence for such a mechanism is provided by the "low ionic strength" crossbridge that was recently characterized in this laboratory. This crossbridge is weakly binding and has a configuration different from that of the rigor (no ATP) crossbridge. The demonstration that attached crossbridges can have different structures is consistent with the idea that contractile force is generated by a transition between two cross-bridge states. (Brenner, Schoenberg, Yu, Podolsky).

Another proposal is based on data from myosin filaments and myofibrils suggesting that the subfragment 2 (S2) moiety of the myosin molecule is "stabilized" when it is closely associated with the backbone of the thick filament, and that it "melts" (and presumably shortens) when it moves away from the backbone (Harrington, 1979). Indications that this process occurs in rabbit psoas fibers have now been obtained from mechanical and structural studies of fibers in the rigor state that were subjected to radial expansion or compression. When ionic strength is lowered and/or pH is raised relative to the physiological value, rigor fibers expand radially (which increases the actin-to-myosin filament spacing) and develop force. In addition, the force-extension curve displaces reversibly along the extension axis, showing that unloaded fibers decrease in length under these conditions. For large amounts of swelling, the increase in filament spacing is sufficient to account for the measured decrease in fiber length through the shortening produced by rotation of S2 around the hinge region of the myosin molecule. However, for smaller amounts of swelling (change in actin-to-myosin filament spacing of 3 nm) the measured decrease in fiber length (about 3 nm) is considerably greater than that attributable to rotation of S2. An additional source of shortening appears to be present in this case. A possible explanation is that fiber swelling is associated with movement of S2 away from the backbone of the thick filament and that this movement causes S2 to decrease in length. (Collett, Podolsky)

Crossbridge detachment rate-constants.

It has been postulated that an unstrained crossbridge should detach, in a muscle fiber, with about the same rate-constant as the soluble myosin fragment, S1, detaches from actin in solution. However, for quite a few conditions, the muscle crossbridges detach significantly slower than S1 in solution. The crossbridge detachment rate-constants are determined from the rate at which strained crossbridges detach from and reattach back to actin in positions of lesser strain. Since they tend to bind crowded together in groups, one possible explanation for the slow relaxation of crossbridge strain could be that crossbridges that detach are retarded from finding suitable reattachment sites in positions of lesser strain due to interference from neighboring crossbridges. This explanation was ruled out by calculation, and experiments are currently underway to see if the relatively slow crossbridge detachment might be related to the double-headedness of the binding of a crossbridge as each crossbridge is composed of the equivalent of two S1 binding sites. (Schoenberg)

Binding of nucleotides and nucleotide analogues to attached crossbridges in muscle fibers and to acto-S1 in solution.

Previous studies in our laboratory of nucleotide and analogue binding to crossbridges in fibers and to acto-S1 in solution with AMPPNP and PP showed equal affinities in the two systems. To further explore this relationship, the binding strength of ADP to attached crossbridges was measured. It was found to be strong, just as strong as the strength of binding of ADP to acto-S1 in solution. The evidence suggests, therefore, that nucleotides and nucleotide analogues bind to attached crossbridges as strongly as to acto-S1 in solutions. This in turn suggests that crossbridge strain does greatly influence analogue or nucleotide binding. (Schoenberg)

Configuration of the low ionic strength crossbridge.

Low angle equatorial X-ray diffraction patterns have been obtained from the relaxed and rigor rabbit psoas fibers at low and normal ionic strengths and at low temperature. In conjunction with modelling, the results suggest that, in the relaxed state, the centers of mass of the myosin heads are not shifted significantly from their detached positions as they become attached. In the rigor state, the tail part of the myosin head moves, probably by a slewing motion, closer towards the thin filament. The structure of the crossbridges in an active muscle could be an intermediate between those found in the relaxed and rigor states. (Yu)

Distribution of mass in frozen hydrated thin sections.

X-ray diffraction and high resolution electron microscopy are complementary techniques for obtaining mass distributions in biological specimens. Muscle fibers that had previously been X-rayed were cryoprotected with sucrose, quickly frozen in liquid ethane, cross-sectioned, and imaged in the electronmicroscope. Distortion produced in the sectioning step was corrected by image processing. Comparison of relaxed and rigor fibers in the region of filament overlap showed larger thin filaments in the latter case, as would be expected from the presence of actomyosin crossbridges. Phase sets for the first five equatorial reflections were obtained by calculation. [Steven (LCDB), McDowall (EMBL), Trus (DCRT), Podolsky]

Fish muscle.

The myofilament lattice in flatfish muscle (plaice, turbot) is more regular than that in mammalian muscle, and it is of interest to know whether the additional order has physiological consequences. Therefore a series of comparative studies were begun. In contrast to rabbit psoas muscle, equatorial X-ray patterns from fin muscle of flatfish showed few crossbridges formed at low ionic strength and 6° C. This probably reflects a difference in kinetic rates in the ATP hydrolysis cycles for the two animals. Secondly, two distinct rigor structures were observed depending on whether the fish muscle was allowed to shorten during the transition into the rigor state. Since the myofilament lattice of the fish is different from those of rabbit muscle and other skeletal muscle, this suggested that the pattern of myosin heads attaching along the thin filaments in a muscle cell depends on the basic lattice structure. (Yu, Squire (Imperial College, London), Podolsky)

Tropomyosin and the low ionic strength crossbridge.

Actomyosin crossbridges form in relaxed fibers at low ionic strength because either (1) the binding of myosin heads to regulated actin is enhanced under this condition, or (2) low ionic strength enables tropomyosin to move away from the "blocking" position on the actin filament. To decide between these alternatives, the tropomyosin-sensitive, actin-based layer lines were studied as a function of ionic strength using a novel two-dimensional, electronic X-ray detector. In stretched fibers, where the actin and myosin filaments do not overlap, low ionic strength did not affect the tropomyosin-sensitive reflections, which supports hypothesis (1). However it remains possible that the binding of tropomyosin to actin is weakened at low ionic strength, making it possible for myosin "heads" to displace the tropomyosin under these conditions. (Collett, Podolsky)

Physiological role of titin and nebulin.

Radiation inactivation analysis showed that the target sizes for loss of active tension and resting tension in rabbit muscle fibers were in the megadalton range and were equal for both phenomena. This finding was followed up using gel electrophoresis and electronmicroscopy. Gels showed that the loss of active and resting tension in irradiated fibers is associated with the degradation of titin and nebulin. The smaller muscle proteins (myosin heavy chain, myosin light chain, actin, actinin, tropomyosin, and troponin) do not change significantly at doses that degrade titin and nebulin. Electronmicroscopy showed that the thick filaments in irradiated resting fibers become disordered in the sarcomere, and that disorder increases when the fibers are activated. The results indicate that the thick filaments are mechanically linked to the nearest Z disc by elastic filaments made of titin and/or nebulin. (Horowits, Kempner, Bisher, Podolsky)

Titin and/or nebulin filaments would be expected to stabilize the positions of the thick filaments in the middle of the sarcomere at sarcomere lengths with sufficient resting tension. This prediction was verified by electronmicroscopy. The critical sarcomere length for A band stability was found to be 2.6 - 2.7 mμ, the sarcomere length where resting tension becomes significant. (Horowits, Podolsky)

Data Analysis Language (Dal)

Dal, an interactive computer language for analyzing scientific data by curve fitting, solving sets of differential equations, and data smoothing, was written and tested. It runs on a PC and makes it possible to perform most basic mathematical manipulations on scalar and array data, to get such data into the program, and to plot the data in various ways. Although it will be used in our laboratory primarily for the analysis of X-ray diffraction patterns, it has general applicability and is expected to be widely used on the NIH campus. (Collett)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 27000-24 LPB
(Formerly
Z01 AM 27000-23 LPB)

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Mechanism of Muscular Contraction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard J. Podolsky	Chief	LPB, NIAMS
Others:	Leepo C. Yu	Research Physicist	LPB, NIAMS
	Brian Collett	Staff Fellow	LPB, NIAMS
	Robert Horowits	Staff Fellow	LPB, NIAMS

COOPERATING UNITS *(if any)* Dr. Alasdair Steven, Laboratory of Cellular and Developmental Biology; Dr. Ellis Kempner, Laboratory of Biochemical Pharmacology; Dr. Alasdair McDowall, European Molecular Biology Laboratory, Heidelberg, W. Germany; Dr. John Squire, Imperial College, London, England.

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Section on Cellular Physics

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 5.5	PROFESSIONAL: 3.5	OTHER: 2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Contractile force in muscle fibers is generated by crossbridges that form between the myosin-containing and actin-containing filaments and hydrolyze ATP. During shortening, the two types of filaments slide relative to each other. Using skinned fiber preparations from rabbit, structural changes associated with crossbridge formation and strain were studied by X-ray diffraction and mechanical methods.

An electronic, position-sensitive, X-ray detector was characterized for quantitative measurement of two-dimensional diffraction patterns and actin-based layer lines were recorded from rabbit fibers at a sarcomere length of 4.0 mμ. The intensities of reflections from the regulatory protein, tropomyosin, were unaffected by ionic strength, showing that formation of the "low ionic strength" actomyosin crossbridge is not due to a direct effect of low ionic strength on the position of tropomyosin on the actin-containing myofilament.

Sarcomere shortening that occurs when rigor (ATP free) fibers are made to expand laterally by decreasing ionic strength and raising pH can generally be accounted for by rotation of the subfragment 2 portion of the actomyosin crossbridge around the backbone of the myosin-containing filament. However, for small lattice expansions an additional source of shortening, which may be related to the physiological force-generating process, appears to be present.

Gel electrophoresis showed that loss of both active tension and resting tension in irradiated muscle fibers is caused by degradation of the megadalton proteins, titin and nebulin, and that the smaller muscle proteins (myosin, actin, tropomyosin, troponin, etc.) remain largely intact. Electronmicroscopy showed that titin and/or nebulin in normal fibers act by positioning the myosin-containing filaments in the middle of the sarcomere.

The overall goal of the project is to find out how the actomyosin system in muscle fibers produces force and motion, and how the mechanical responses are regulated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 27001-12 LPB
(Formerly
Z01 AM 27001-11 LPB

PERIOD COVERED October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Contractility and Excitability of Skeletal and Cardiac Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Mark Schoenberg, Medical Officer, LPB, NIAMS

COOPERATING UNITS *(if any)*
Dr. Evan Eisenberg, Laboratory of Cell Biology, NHLBI
Dr. Aydin Tözeren, Dept. of Mechanical Engineering, The Catholic University,
Washington, D.C.

LAB/BRANCH
Laboratory of Physical Biology

SECTION

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In FY '84 we reported that the binding constant for AMP-PNP and PPi binding to attached crossbridges in fibers was considerably weaker than that reported for binding to acto-S1 in solution. New biochemical evidence, however, suggests that the strength of binding of AMP-PNP and PPi to acto-S1 is similar to that obtained in our fiber experiments. To further explore the relationship between the fiber and solution binding constants for nucleotide and analogue, in FY '86 we measured the binding of ADP to attached crossbridges. We found it to be moderately strong, about as strong as the binding of ADP to acto-S1 in solution. On balance, therefore, the evidence suggests the binding of nucleotides and the nucleotide analogues to crossbridges is about as strong as that to acto-S1 in solution.

We have also measured the crossbridge detachment rate-constants in the fiber and compared them with those for detachment of S1 from actin in solution. We detect crossbridge detachment by measuring the rate of decay of force after rapid stretch of a single isolated muscle fiber. This is possible since the decay of force after stretch is due to strained crossbridges detaching and then reattaching in positions of lesser strain. We find that in the fiber the decay of force following a stretch is not described by a single rate-constant but by multiple rate-constants spanning a wide range. The fastest rate-constants are comparable to the detachment rate-constant of S1 from actin in solution but the majority are much slower. In FY '86 we ruled out the possibility that this slowness in apparent detachment rate was related to crossbridge crowding. Currently, experiments are underway exploring other posssible explanations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM-27002-23 LPB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sense of Rhythm

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John B. Buck, Research Physiologist, Laboratory of Physical Biology, NIAMS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is inactive at the present time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM-27003-17 LBP

PERIOD COVERED October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The dynamic properties of cell membranes and related systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Norman L. Gershfeld, Research Chemist, Laboratory of Physical Biology, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to Laboratory of Biochemical Pharmacology, NIDDK.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM-27004-16 LPB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physical Chemistry of Cell Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Melvin H. Gottlieb, Research Chemist, Laboratory of Physical Biology, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is presently inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM-27006- 29 LPB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental biochemistry of insect metamorphosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Leo Levenbook, Research Chemist, Laboratory of Physical Biology, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Laboratory of Cellular and Developmental Biology, Section on Structural Biology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM-27008-27 LPB

PERIOD COVERED October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biophysical studies of metabolic activity and control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ellis S. Kempner, Physicist, Laboratory of Physical Biology, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Laboratory of Biochemical Pharmacology, NIDDK.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 27009-08 LPB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Macromolecular Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Alasdair C. Steven, Visiting Scientist, LPB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Laboratory of Cellular and Developmental Biology, Section on Structural Biology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AR 27012-02 LPB (Formerly Z01 AM 27012-01 LPB)

PERIOD COVERED October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural and Mechanical Properties of Skeletal Muscle Fibers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Leepo C. Yu, Research Physicist, Laboratory of Physical Biology, NIAMS

COOPERATING UNITS *(if any)*
Dr. Bernhard Brenner (University of Tübingen, FRG)
Dr. John Squire, (Imperial College, London, U.K.)

LAB/BRANCH
Laboratory of Physical Biology

SECTION

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In a skeletal muscle, acto-myosin crossbridges, i.e. binding of myosin heads with the thin filaments, are formed under a wide range of conditions, such as in the presence of ATP and Ca^{++} (contracting state), without Ca^{++} (relaxed state), in the absence of ATP (rigor state) and in the presence of ATP analogues. Our goal is to characterize the structural, and simultaneously mechanical properties of the crossbridges under these conditions by studying X-ray diffraction patterns and mechanical measurements from single muscle fibers.

Low angle equatorial X-ray diffraction patterns have been obtained from the relaxed and rigor rabbit psoas fibers at low and normal ionic strengths and at low temperature. In conjunction with modelling, the results suggested that in the relaxed state, the centers of mass of the myosin heads are not shifted significantly from their detached positions as they become attached. In the rigor state, the tail part of the myosin head moves, probably by a slewing motion, closer towards the thin filament. The structure of the crossbridges in an active muscle could be an intermediate between those found in the relaxed and rigor states. Further work is in progress to obtain high resolution diffraction patterns from actively contracting single muscle fibers.

By conducting comparative studies, further understanding of the molecular structure associated with actomyosin interaction could be achieved. In contrast to rabbit psoas muscle, the fin muscle from flatfish showed few crossbridges formed at low ionic strength and 6°C. This probably reflects a difference in kinetic rates in the ATP hydrolysis cycles for the two animals. Secondly, two distinct rigor structures were observed depending on whether the fish muscle was allowed to shorten during the transition into the rigor state. Since the myofilament lattice of the fish is different from those of rabbit muscle and other skeletal muscle, this suggested that the pattern of myosin heads attaching along the thin filaments in a muscle cell depends on the basic lattice structure.

Annual Report of the Arthritis and Rheumatism Branch
National Institute of Arthritis and Musculoskeletal and Skin Diseases

The Branch's professional activities include both clinical and laboratory investigations. The clinical involvement is substantial: During the past year there were some 300 admissions (average length of stay 14 days) accounting for over 4,000 patient-days. Over 36000 follow-up outpatient visits were scheduled including approximately 100 evaluations of new patients. In addition, ARB physicians responded to some 100 requests for consultation at the Clinical Center. The clinical investigations involve the collection and study of tissue and blood specimens as well as trials of various therapeutic regimens, chiefly on patients with systemic lupus erythematosus but also on those with rheumatoid arthritis and polymyositis. In order to assure the smooth functioning of this heavy clinical program, Dr. John H. Klippel serves as Clinical Coordinator for the Branch.

The laboratory investigations involve studies on animal models of lupus, arthritis and polymyositis as well as fundamental studies at the cellular and molecular level, on various aspects of the immune response and inflammatory processes.

I. Disease Related Studies

A. Rheumatoid Arthritis

1. Pathogenesis

a. Animal Models (48)*

Chronic proliferative and erosive synovitis, as well as hepatic granulomas, can be induced in susceptible rats by systemic administration of aqueous suspensions of cell wall fragments from selected bacteria, such as group A, B or C streptococci and _Lactobacillus casei_. The arthritis resembles human rheumatoid arthritis in its clinical, pathological and immunological characteristics. The development of acute and chronic disease is a direct consequence of the deposition and

*Numbers in parentheses refer to last digits of project number, e.g. Z01 AR 41048 persistence of cell wall fragments in hepatic and synovial tissues. Comparative studies in athymic-euthymic and cyclosporin A treated/control rats have demonstrated that the early acute phases of disease are independent of the thymus and T-lymphocytes, whereas the chronic phases are thymus-dependent. Chronic disease is characterized by tissue infiltration with lymphocytes bearing T helper/inducer cell surface markers. Interestingly, bone and cartilage destruction appears to be mediated by a "transformed" appearing fibroblast-like cell. Proliferation, and bone destruction mediated by the fibroblast-like cell is dependent upon T-lymphocyte derived cytokines. The destructive process in inhibited by cyclosporin A and the retinoid, 4-hydroxyphenyl retinamide. The development of disease is paralleled by enhanced tissue expression of class II-major histocompatibility antigens. Enhanced expression of these antigens is not observed in resistant rat strains. This animal model provides a powerful tool to investigate mechanisms that regulate susceptibility to chronic inflammation, as well as mechanisms involved in produced tissue destruction.

b. Human Disease (66)

Synovial tissues and peripheral blood mononuclear cells are obtained from patients with active chronic synovitis, such as rheumatoid arthritis. The analysis of these samples suggests that conditions such as rheumatoid arthritis comprise an immunopathologic spectrum. Patients at the polar extremes of this spectrum display consistent phenotypic and functional characteristics. An anergic subpopulation of patients with rheumatoid arthritis has been compared with a clinically similar nonanergic group. Peripheral blood mononuclear cells from anergic-patients in general exhibited lower T helper/inducer to T suppressor lymphocyte ratios and increased frequencies of HLA-DR bearing T lymphocytes than those from nonanergic patients. In contrast, synovial tissues from anergic patients in general had high intensity T helper/inducer lymphocyte infiltration, while tissues from the nonanergic group were, in general, sparsely infiltrated by lymphocytes. Moreover, synovial mast cell numbers were closely correlated with the intensity of T cell infiltration, i.e., the more dense the T-cell infiltration, the more numerous were the mast cells. Data also suggested that these phenotypic differences are also reflected by cytokine secretion (e.g., T lymphocyte derived chemotactic factor is produced in abundance by synovium from anergic, but not nonanergic patients). These findings provide insight into pathogenic mechanisms and may result in improved therapeutic procedures.

2 Therapeutic trials

a. The efficacy and toxicity of daily, oral cyclosporin A (CSA) in patients with active rheumatoid arthritis is being evaluated in a 6 month double-blind study of low dose (1mg/kg/day) versus high dose (10mg/kg/day) CSA. Toxicity is monitored weekly for the first 2 months and then every 2 weeks for 6 months. Disease activity is assessed on a bi-monthly basis. Patients who do not develop at least a 40% improvement in disease activity at 6 months are dropped from the study, while those with a 40% or greater response continue for an additional 6 months of drug therapy. The effects of CSA on various measures of T lymphocyte function in peripheral blood and within the synovium is studied in all patients.

To date (July, 1986), the enrollment phase of the project has been completed and a total of 30 patients have entered the trial. All patients will complete the 6 month follow-up by November, 1986. A detailed analysis of the clinical toxicity and immunologic data will be done. Patients with a successful clinical outcome defined by by an improvement of 40%, or greater, in the articular index will be studied for a maximum of one year of drug therapy.

b. A pilot study is evaluating high dose intravenous methotrexate (500mg/meter square) with leucovorin rescue in the management of patients with severe, active rheumatoid arthritis. Patients must have failed at least one second line agent or high dose corticosteroid therapy to be entered. Methotrexate and leucovorin are given every 2 weeks for a total of 4 doses. Patients are evaluated during this period and for an additional 4 months for changes in arthritis activity and complications related to therapy. The effects of high-dose methotrexate on various measures of lymphocyte function and cell populations in the peripheral blood is studied.

Courses of methotrexate-leucovorin have been completed in 5 patients. Each patient has experienced an impressive improvement in the level of arthritis activity with a prompt reduction in articular index of 50%, or greater, in all patients at 6 weeks of study. Clinical improvement has been associated with a marked reduction of peripheral blood T lymphocyte DR expression. Relapse of the disease has been observed in two of the three patients followed beyond the treatment course.

Data obtained from this study is currently being analyzed and a manuscript detailing the findings is in preparation.

Consideration is being given to further evaluation of high-dose methotrexate in rheumatoid arthritis by a formal randomized, controlled clinical trial.

B. Systemic Lupus Erythematosus (SLE)

1. Pathogenesis

a. Animal models (20)

Autoimmunity in New Zealand mice has a genetic and immune basis; however, expression of severity can be modified by viral and other environmental factors as well as sex hormones. The immune factors are complex. These include excessive B-cell activation and abnormal T cell function.

The CBA/N X-linked gene (xid) is completely expressed in congenic mice including such autoimmune congenic mice bearing xid as NZB.xid, NZW.xid, (NZB x NZW)F_1xid, BXSB.xid, (NZB x BXSB)F_1.xid and (NZW x BXSB)F_1.xid. Such mice fail to make anti-DNA, anti T cell antibodies, and anti-RBC antibodies. They are protected from renal and cardiac disease and premature death.

NZB.xid congenic mice, which do not have autoantibodies and which do not get sick, manifest the T cell defects seen in NZB mice. This result suggests that the T cell defects do not result from the B cell hyperactivity.

Consistent with the latter, MRL-lpr/lpr.xid and C3H/HeJ-gld/gld.xid mice have a marked reduction in autoantibody production but unchanged T cell lymphadenopathy.

Mice not destined to develop overt clinical autoimmune disease develop rapidly progressive autoimmune disease and premature death when administered polyclonal B cell activators.

Polyclonal B-cell activators induce amyloidosis. This occurs in mice bearing CBA/N X-chromosomes indicating that the B-cell subset controlled by xid is not necessary for amyloid production.

Analysis of recombinant inbred mice produced by mating NZB with non-autoimmune mice indicates that in NZB mice there is no "autoimmunity gene," rather, the full expression of illness requires at least six separate genes. However, the presence of fewer genes allows for the expression of milder degrees of autoimmunity and individual autoantibodies. Studies of linkage of specific genes and individual traits indicate that one gene may be necessary, but not sufficient for the expression of more than one autoimmune trait. The traits of NZB mice are divisible into 2 families of unlinked genes, one of which underlies anti-DNA, anti-RBC, anti-MULV and abnormal tolerance and the other of which underlies NTA, increased IgM, MULV expression, and increased stem cell activity.

The information for the tolerance defect in NZB mice is contained in the NZB marrow pre-T cell. NZB cells prevent the development of tolerance by normally tolerizable DNA/2 cells. Therefore, the tolerance defect is not a passive failure to become tolerant, but an active interference with tolerance.

Normal mice fed egg albumin (OVA) or bovine gamma globulin (BGG) are tolerized so that subsequent injections of these antigens induce a reduced antibody response. Autoimmune NZB mice are defective in gastrointestinal tolerance to both OVA and BGG. Thus, defective gastrointestinal tolerance may contribute importantly to the autoimmune disease of humans with systemic lupus, especially in view of the presence of anti-BGG in the sera of some patients. Eating certain foods could trigger or perpetuate a disease flare.

Autoimmune mice have reduced interleukin 2 production following stimulation with Con A. This defect is largely corrected by adding phorbol myristic acetate as a stimulant.

Whereas in normal mice immune interferon is mostly produced by Ly 2^+ cells, in MRL-lpr/lpr mice it is produced especially by Ly 1^+, 2^- cells.

T cell lines can be established from MRL-lpr/lpr spleens. These lines can produce IL 2 or interferon *in vitro* and respond to stimulation by Ia^+ non-T cells. Some can help B cells to differentiate into Ig secreting cells. The growth of these lines is inhibited by pre-cultured macrophages plus anti-Ia antibodies.

Sera from NZB mice will allow a subimmunogenic dose of SRBC to be antigenic in normal mice. Therefore, an immune enhancer may contribute to autoimmunity.

Autoimmune mice manifest differential oncogene expression. *lpr*/*lpr* and *gld*/*gld* mice have increased *myb* and *raf* mRNA.

The xid gene prevents ras and myc expression but not myb.

b. Human disease. Study of T-lymphocytes (23)

i. Cells and cell markers

Studies with monoclonal antibodies, which identify T cell subsets, indicate that some patients with active SLE have a marked decrease in suppressor cells and suppressor cell precursors. Other patients have an increase in suppressor cells relative to helper cells. In most cases of abnormal T suppressor/T helper ratios, the abnormal ratio stems from a quantitative defect in helper cells rather than suppressor cells. Most importantly, the ratio correlated with clinical features of SLE. Hgh suppressor:helper ratios are associated with renal disease, thrombocytopenia, and lymphopenia. In contrast, patients with relatively fewer suppressors than normal tend to have a multisystem disease with muscle disease, lymphadenopathy, the sicca syndrome, and older age of onset of disease. Symptom clusters are also appreciated without the need for T cell phenotyping.

Proto-oncogene expression is abnormal in SLE patients. Those associated with B cell activation have increased expression in patients with active disease, and also in patients with other autoimmune rheumatic diseases.

Angioimmunoblastic lymphadenopathy is characterized by excessive helper T cell function with essentially normal B cells.

ii. Anti DNA antibodies

Antibodies to nucleic acids are important diagnostically and pathogenetically in systemic lupus. Patients with active SLE have an increase in DNA in immune complexes. Such DNA is enriched in guanine (G) and cytosine (C). Low molecular weight DNA enriched in G-C is released from SLE leukocytes. This DNA may be important in the pathogenesis of SLE. Genes favoring the production of anti-DNA may include those which lead to the release of immunogenic DNA.

DNA will be isolated from the immune complexes of patients with lupus and the sequences determined after cloning in plasmids.

Because DNA anti-DNA complexes may be important in the pathogenesis of lupus nephritis, identifying the nature of the DNA may give a clue as to how it has come to be bound to anti-DNA antibodies. In particular, we wish to determine whether any viral or other non-human DNA is present, and whether any particular DNA from the human is preferentially found.

2. Therapeutic studies

a. Animal models (22)

A diet enriched in a polyunsaturated fatty acid, eicosapentaenoic acid, markedly retards the development of autoimmunity in (NZB x NZW) F1 females. This works even after the onset of disease.

Cyclophosphamide is effective treatment in a variety of autoimmune strains unrelated to the cellular basis of illness or the nature (B or T cell) of the proliferating cells. This therapy is effective against the lymphadenopathy of MRL-*lpr*/*lpr* and C3HeJ-*gld*/*gld* mice and the coronary artery disease of BXSB and (NZW x BXSB)F1 mice as well as the renal disease of most examples of murine lupus. Cyclophosphamide markedly reduces the elevated *myb* RNA in MRL-*lpr*/*lpr* mice lymph nodes and increases the very low thymic *myb* expression.

Cyclosporin A, a drug which interferes with T cell function, is effective treatment for the arthritis, nephritis, and lymphoproliferation of MRL-*lpr*/*lpr* mice.

b. Human diseases (40)

The Arthritis and Rheumatism Branch has a major interest in the management of the nephritis of systemic lupus erythematosus (SLE). Renal failure is the most feared complication in the disease. Ths renal damage is largely in the glomeruli. It has been suggested that drugs capable of altering the antibody response, "immunosuppressives", would reduce the formation of antibody and thus of antigen-antibody complexes. The objective of this study is to assess the efficacy and toxicity of various immunosuppressive drug programs in SLE nephritis.

Long-term follow-up shows that separately oral cyclophosphamide (CY) and oral azathiprine (ZA) are of only minimal benefit overall; however, a subgroup of patients with minimal chronic changes on biopsy respond.

Young patients (8-23 years of age) are at greater risk of developing renal failure than are older >24 years of age) patients with SLE and nephritis.

The data suggest that oral CY is less effective and more toxic than bolus CY.

Long-term follow-up demonstrates a substantial and highly significant benefit of cyclophosphamide over corticosteroids. This was only apparent beyond the 5 year follow-up point.

C. Polymyositis

1. Etiology/Pathogenesis (69)

Characteristic antibodies which react with normal tissues (autoantibodies) develop in patients with a number of diseases and in some animals. Considerable evidence suggests that some of these antibodies arise because of specific interactions between an exogenous agent (virus, bacterium, drug) and the sites to which the antibody binds. A search is underway to determine whether certain picornaviridiae can be implicated in the causation of inflammatory myositis and the characteristic myositis related antibodies which react with amino acid t-RNA synthetases.

A rapid, high yield partial purification of hisitdyl-tRNA synthetase has been achieved.

A specific ELISA has been developed and used to survey a large number of sera from patients with and without polymyositis.

A careful study of the virus-induced myositis has defined its pathology, time course, strain dependence.

In situ hybridization of the tissues of infected mice is underway with a probe for EMC 221A made in this lab.

Probes for Coxsackie and adenoviruses are being used for *in situ* hybridization probing of human biopsy specimens.

Human monoclonal antibodies to histidyl-tRNA synthetase have been made from EBV-transformed cell lines.

2. Therapeutic trials (71)

Severe polymyositis or dermatomyositis which responds poorly to corticosteroids may be difficult to treat. Because of anecdotal reports suggesting efficacy, we are comparing plasmapheresis and leukapheresis with a sham procedure. Patients who fail to respond will be offered pulse intravenous cyclophosphamide.

II. Fundamental Studies (25, 51)

The IgE-Mast cell system is of significance in the pathophysiology of allergy, parasitic infections, and inflammation. It is also an experimentally convenient system to study mechanisms of antigen-induced antibody-mediated cellular reactions in general. Finally, it is an excellent model of the study of exocytosis. So far we are continuing to make good progress in the elucidation of the molecular mechanismm of this system. Knowledge of these may eventually permit rational intervention in situations where the system contributes to pathology.

During the past year the following subjects were focussed on:

1) Continued characterization of the subunits of the receptor. In particular, additional studies were performed on: The isoelectric point, size, and multiplicity of the gamma chains of the alpha, beta, $(\text{gamma})_n$ complex. Completing a compositional analysis of each of the chains using both in vivo incorporation protocols and amino acid analysis. Examining the relationship between the subunits using analysis by high pressure liquid chromatography of tryptic and chymotryptic digests of each of the chains. 2) Continued attempts were made to obtain peptides in sufficient quantities to perform sequence analysis in preparation for making cDNA probes. Continued attempts were made to generate monoclonal antibodies which can be used to probe the topology of the peptides in the plasma membrane. 4) Functional studies during the past year have focussed on attempts to analyze the hydrolysis of (poly)phosphosinositides that occurs as a consequence of aggregation of the receptors for IgE. In particular, various types of altered cells or cell-free preparations have been examined such as saponized cells, cytoblasts, and membrane preparations.

An attempt is being made to understand the mechanisms by which the receptors are incorporated into the plasma membrane and after stimulation, internalized. Advantage is taken of the fact that some of the same receptors that are not specifically aggregated can be followed simultaneously as can receptors for other Ig such as IgG. The principal findings during the current period has been evidence that there are cellular mechanisms by which the different receptors and receptor configurations can be discriminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41018-12 ARB

PERIOD COVERED

October 1, 1985 to January 1, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Production of covalently cross-linked immune complexes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Paul H. Plotz	Senior Investigator	ARB/NIAMS
	LesLie Schrieber	Visiting Associate	ARB/NIAMS
	William Mullins	Med. Staff Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41020-19 ARB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Autoimmunity in Mice with SLE-like Illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, NIAMS
Michael F. Seldin, Medical Staff Fellow, Cellular Immunology Section, NIAMS
Daniel L. Kastner, Medical Staff Fellow, Cellular Immunology Section, NIAMS
John D. Mountz, Medical Staff Fellow, Cellular Immunology Section, NIAMS
William C. Gause, Staff Fellow, Cellular Immunology Section, NIAMS
Jeffrey N. Siegel, Medical Staff Fellow, Cellular Immunology Section, NIAMS
Igal Kedar, Visiting Associate, Cellular Immunology Section, NIAMS

COOPERATING UNITS *(if any)*

David I. Cohen, Senior Investigator, NIDDK
Lyn Yaffe, Pathologist, NMRI, Bethesda, MD
J. F. Mushinski, Senior Investigator, NCI

Foreign: None

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Z01 AM 41020-19 ARB

Autoimmunity in New Zealand mice has a genetic and immune basis; however, expression of severity can be modified by viral and other environmental factors as well as sex hormones. The immune factors are complex. These include excessive B-cell activation and abnormal T cell function.

A single gene, xid, prevents disease in congenic NZB and (NZB x NZW) F1 and BXSB mice, and markedly retards disease in MRL-lpr/lpr mice. BXSB mice have a Y chromosome-linked gene which accelerates autoimmunity in autoimmune mice, but does not cause it in non-autoimmune mice. This gene requires an autosomal gene from BXSB mice for full expression in NZB mice, but not in NZW mice.

All of the autoimune strains studied have a defect in experimental tolerance. In NZB mice, the defect maps to a pre-T stem cell. Small numbers of NZB T-cells interfere with the development of tolerance suggesting that they secrete a factor which causes lymphocyte activation rather than supression.

Analysis of cytokines produced by autoimmune mice has led to a first level of appreciation of the importance of low molecular weight mediators of cellular communication in the immune abnormalities of autoimmune mice. The defects in autoimmune mice can be mimicked in normal mice by appropriate manipulation (removal of regulatory T cell function and chronic immune stimulation).

Studies of gastrointestinal immunity reveal that ingestion of certain food may fail to induce tolerance normally or even induce an immune response. Therefore, food in the diet of certain patients could trigger immune responses.

Different autoimmune mice have increased expression of different oncogenes. lpr/lpr and gld/gld mice have increased myb and T cell receptor (Beta gene). The xid gene prevents increased myc and ras expression, suggesting that that oncogene is associated with the Lyb 5 plus subset and autoantibody production. The studies of gene expression in lpr/lpr mice have pointed to a new pathway for T cell maturation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41021-18 ARB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antinuclear Antibodies in Spontaneous and Drug-induced SLE and Other Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Alfred D. Steinberg, Chief, Cellular Immunology Section, NIAMS
Curtis L. Scribner, Medical Staff Fellow, Cellular Immunology Section, NIAMS
Yoshiaki Ishigatsubo, Visiting Fellow, Cellular Immunology Section, NIAMS
Dennis M. Klinman, Senior Medical Staff Fellow, Cellular Immunology Section, NIAMS

COOPERATING UNITS *(if any)*
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C. Morimoto, Harvard Medical School
Foreign: None

LAB/BRANCH
Arthritis and Rheumatism

SECTION
Cellular Immunology

INSTITUTE AND LOCATION
NIAMS - Bldg. 10, Rm. 9N-218, Bethesda, Md. 20892

TOTAL MAN-YEARS: 5	**PROFESSIONAL:** 3	**OTHER:** 2

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Z01 AM 41021-18 ARB

Antibodies to nucleic acids are important diagnostically and pathogenetically in systemic lupus. Patients with active SLE have an increase in DNA in immune complexes. Such DNA is enriched in guanine (G) and cytosine (C). Low molecular weight DNA enriched in G-C is released from SLE leukocytes. This DNA may be important in the pathogenesis of SLE. Genes favoring the production of anti-DNA may include those which lead to the release of immunogenic DNA.

In mice a subset of B cells is responsible for anti-DNA production. In NZB mice, B cells can transfer anti-DNA to congenic NZB.xid recipients. This transfer is prevented by treating either donors animals or recipients with anti-Ia antibodies. Similar approaches to human disease are being sought.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AR 41022-14 ARB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Therapeutic Studies in Murine Lupus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, NIAMS
John D. Mountz, Medical Staff Fellow, Cellular Immunology Section, NIAMS

COOPERATING UNITS *(if any)*

D. Robinson, Harvard Medical School

Foreign: None

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Z01 AM 41022-14 ARB

A diet enriched in a polyunsaturated fatty acid, eicosapentaenoic acid, markedly retards the development of autoimmunity in (NZB x NZW) F1 females. This works even after the onset of disease. Human trials are being carried out.

Cyclophosphamide is effective treatment in a variety of autoimmune strains unrelated to the cellular basis of illness or the nature (B or T cell) of the proliferating cells. This therapy is effective against the lymphadenopathy of MRL-lpr/lpr and C3H/HeJ-gld/gld mice and the coronary artery disease of BXSB and (NZW x BXSB)F1 mice as well as the renal disease of most examples of murine lupus. Cyclophosphamide markedly reduces the elevated myb RNA in MRL-lpr/lpr mice lymph nodes and increases the very low thymic myb expression.

Cyclosporin A, a drug which interferes with T cell function, is effective treatment for the arthritis, nephritis, and lymphoproliferation of MRL-lpr/lpr mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41023-12 ARB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Immune Abnormalities in Patients with Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Alfred D. Steinberg, Chief, Cellular Immunology Section, NIAMS
Dennis M. Klinman, Medical Staff Fellow, Cellular Immunology Section, NIAMS
LeeAnn Tracy, Guest Researcher, Cellular Immunology Section, NIAMS
Yoshiaki Ishigatsubo, Visiting Fellow, Cellular Immunology Section, NIAMS

COOPERATING UNITS *(if any)*
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Yves Borel, Harvard Medical School
Foreign: None

LAB/BRANCH
Arthritis and Rheumatism

SECTION
Cellular Immunology

INSTITUTE AND LOCATION
NIAMS - Bldg. 10, Rm. 9N-218, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Z01 AM 41023-12 ARB

Patients with SLE have excessive B cell proliferation. They have increased proliferation of bone marrow stem cells and circulating B cell precursors. Disease activity is characterized by a switch from just proliferation to differentiation and immunoglobulin synthesis. In some patients the T cell regulation of B cell functions is abnormal. In an attempt to better understand cell-cell inter-actions, normals were studied for the capacity of T cells to respond to autologous non-T cells. IL 2 production by T4 plus cells was necessary for the proliferation of T8 plus (suppressor) cells. This function is subnormal in patients with SLE.

Monoclonal antibodies were used to study subpopulations of SLE patients. One subset, with a reduced ratio of helper/suppressor cells, was characterized clinically by renal disease, thrombocytopenia, leukopenia and early age of onset of disease. Another subset had a high ratio and a clinical picture which included muscle and lung disease, Sicca syndrome, lympadenopathy and CNS disease. Thus, SLE may not be a single disease, but a group of syndromes with different genetic and cellular bases.

SLE patients have a variety of T cell defects. These are largely explained by anti-T cell antibodies produced by the B cells of these patients.

The more rapidly progressive autoimmune lymphoproliferative disorder, angioimmunoblastic lymphadenopathy is characterized by markedly effective helper T cells which drive B cells to proliferate and differentiate. The B cells themselves are normal in this variant.

Proto-oncogene expression is abnormal in patients with SLE. There is a significant increase in expression characteristic of activated B cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41025-15 ARB
(formerly
Z01 AM 41025-14 ARB)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the cell surface receptor for IgE

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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G. Alcaraz	Guest Researcher	ARB/NIAMS		
J.P. Kinet	Visiting Associate	ARB/NIAMS	M. Bond	DNAX
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V. Pridluda	Visiting Fellow	ARB/NIAMS	M. Beaven	LCP/NHLBI
S. Dreskin	Guest Researcher	ARB/NIAMS	T. Sullivan	Wash. Univ.
T. Jin Kim	Expert	ARB/NIAMS		

COOPERATING UNITS *(if any)*
LCP/NHLBI LBP/NIDDK DNAX Foreign: None
LTB/C FDA Dept. Int. Med., Wash. University

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	8	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are pursuing an in-depth investigation on the structure and function of the plasma membrane receptor for immunoglobulin E. (IgE). Most of the work utilizes a cell line called rat basophilic leukemic cells recently found to represent a tumor analog of mucosal-type mast cells. During the past year we have focussed on the following subjects: 1) Continued characterization of the subunits of the receptor. In particular, we performed additional studies on the isoelectric point, size, and multiplicity of the gamma chains of the alpha, beta, (gamma)n complex. We have essentially completed a compositional analysis of each of the chains using both in vivo incorporation protocols and amino acid analysis. We also examined the relationahip between the subunits using analysis by high pressure liquid chromatography of tryptic and chymotryptic digests of each of the chains. 2) Continued attempts were made to obtain peptides in sufficient quantities to perform sequence analysis in preparation for making cDNA probes. 3) Continued attempts were made to generate monoclonal antibodies which can be used to probe the topology of the peptides in the plasma membrane. 4) Functional studies during the past year have focussed on attempts to analyze the hydrolysis of (poly)phosphosinositides that occurs as a conseuqence of aggregation of the receptors for IgE. In particular, various types of altered cells or cell-free preparations have been examined such as saponized cells, cytoblasts, and membrane preparations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41040-14 ARB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of various cytotoxic drug programs in diffuse lupus neprhitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, NIAMS
Paul H. Plotz, Senior Investigator, ARB, NIAMS
John H. Klippel, Senior Investigator, ARB, NIAMS
Ronald L. Wilder, Senior Investigator, ARB, NIAMS

COOPERATING UNITS *(if any)*

James E. Balow, Senior Investigator, ARB, NIAMS
Howard A. Austin, Attending Nephrologist, Clinical Center

Foreign: None

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIAMS - Bldg. 10, Rm. 9N-218, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Z01 AM 41040-14 ARB

Patients with the diffuse forms of systemic lupus erythematosus nephritis previously entered into therapeutic trials continue to be evaluated. Long-term studies suggest that the degree of scarring on the renal biopsy may provide important prognostic information. The subset of patients with a mild degree of scarring responds to oral azathioprine or oral cyclophosphamide when added to low doses of corticosteroids. Patients with a greater degree of scarring are not effectively treated with those drug programs, but may respond to intravenous boluses of cyclophosphamide. The relative effectiveness and toxicity of various drug programs continues to be systematically evaluated. Patients aged 8-23 years are at greater risk of developing renal functional deterioration than are older patients with SLE and nephritis. Long-term follow-up of patients with lupus nephritis demonstrate clear-cut benefit of immunosuppressive-cytotoxic drugs over conventional corticosteroid therapy. The benefit was greatest with cyclophosphamide. The differences are highly significant, but only are apparent when follow-up is substantially beyond the 5 year point.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41048-07 ARB
(formerly
Z01 AM 41048-06 ARB)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bacterial Cell Wall-Induced Arthritis and Hepatic Granuloma Formation in the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.L. Wilder, Senior Investigator, ARB/NIAMS

COOPERATING UNITS *(if any)*
Laboratory of Microbial Immunology, NIDR   Foreign: None
Laboratory of Chemoprevention, NCI

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIAMS/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects   ☐ (b) Human tissues   ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chronic proliferative and erosive synovitis, as well as hepatic granulomas, can be induced in susceptible rats by systemic administration of aqueous suspensions of cell wall fragments from selected bacteria, such as group A, B or C streptococci and Lactobacillus casei. The arthritis resembles human rheumatoid arthritis in its clinical, pathological and immunological characteristics. The development of acute and chronic disease is a direct consequence of the deposition and persistence of cell wall fragments in hepatic and synovial tissues. Comparative studies in athymic/euthymic and cyclosporin A treated/control rats have demonstrated that the early acute phases of disease are independent of the thymus and T-lymphocytes; whereas, the chronic phases are thymus-dependent. Chronic disease is characterized by tissue infiltration with lymphocytes bearing T helper/inducer cell surface markers. Interestingly, bone and cartilage destruction appears to be mediated by a "transformed" appearing fibroblast-like cell. Proliferation, and bone destruction mediated by the fibroblast-like cell is dependent upon T-lymphocyte derived cytokines. The destructive process is inhibited by cyclosporin A and the retinoid, 4-hydroxyphenyl retinamide. The development of disease is paralleled by enhanced tissue expression of class II- major histocompatibility antigens. Enhanced expression of these antigens is not observed in resistant rat strains. This animal model provides a powerful tool to investigate mechanisms that regulate susceptibility to chronic inflammation, as well as mechanisms involved in tissue destruction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41051-07 ARB
(formerly
Z01 AM 41051-06 ARB)

PERIOD COVERED
October 1 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural history and in situ organization of the receptors for IgE

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. C. Isersky Carter Senior Investigator (thru 1/86)
H. Metzger Chief, ARB/NIAMS (from 2/86)

C. Ra	Visiting Fellow	ARB/NIAMS
K. White	Visiting Fellow	ARB/NIAMD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Aggregation of the receptors with high affinity for immunoglobulin E (IgE) on mast cells and related cells by antigen leads to release of granules (exocytosis) and their contents. In addition, the aggregated receptors are internalized and they, the IgE bound to them and the antigen are degraded. In this project an attempt is being made to understand the mechanisms by which the receptors are incorporated into the plasma membrane and after stimulation, internalized. Advantage is taken of the fact that some of the same receptors that are not specifically aggregated can be followed simultaneously as can receptors for other Ig such as IgG. The princpipal findings during the current period has been evidence that there are cellular mechanisms by which the different receptors and receptor configurations can be discriminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41066-04 ARB
(formerly
Z01 AM 41066-03 ARB)

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Characterization of Synovial Tissue from Patients w/RA & Related Cond.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.L. Wilder, Senior Investigator, ARB/NIAMS

COOPERATING UNITS *(if any)*

Laboratory of Microbial Immunology, NIDR
Laboratory of Pathology, NCI

Foreign: None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews

☒ (b) Human tissues

☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Synovial tissues and peripheral blood mononucler cells are obtained from patients with active chronic synovitis, such as rheumatoid arthritis. The analysis of these samples suggests that conditions, such as rheumatoid arthritis, comprise an immunopathologic spectrum. Patients at the polar extremes of this spectrum display consistent phenotypic and functional characteristics. An anergic subpopulation of patients with rheumatoid arthritis has been compared with a clinically similar nonanergic group. Peripheral blood mononuclear cells from anergic patients, in general, exhibited lower T helper/inducer to T suppressor lymphocyte ratios and increased frequencies of HLA-DR bearing T lymphocytes than those from nonanergic patients. In contrast, synovial tissues from anergic patients, in general, had high intensity T helper/inducer lymphocyte infiltration, while the tissues from the nonanergic group was, in general, sparsely infiltrated by lymphocytes. Moreover, synovial mast cell numbers were closely correlated with the intensity of T cell infiltration, i.e., the more dense the T-cell infiltration, the more numerous were the mast cells. Data also suggested that these phenotypic differences are also reflected by cytokine secretion (e.g., T lymphocyte derived chemotactic factor is produced in abundance by synovium from anergic, but not from nonanergic patients). These findings provide insight into pathogenic mechanisms and may result in improved therapeutic procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41069 03 ARB
(formerly
Z01 AM 41069-02 ARB)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The role of viruses in the induction of autoantibodies in humans and mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Paul H. Plotz	Senior Investigator	ARB/NIAMS
	F.W. Miller	Medical Staff Fellow	ARB/NIAMS
	T. Biswas	Visiting Fellow	ARB/NIAMS
	M. Cronin	Medical Staff Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*
Laboratory of Oral Medicine, NIDR   Foreign: None

LAB/BRANCH
Arthritis and Rheumatism

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects   ☐ (b) Human tissues   ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Characteristic antibodies which react with normal tissues (autoantibodies) develop in patients with a number of diseases and in some animals. Considerable evidence suggests that some of these antibodies arise because of specific interactions between an exogenous agent (virus, bacterium, drug) and the sites to which the antibody binds. A search is underway to determine whether certain picornaviridiae can be implicated in the causation of inflammatory myositis and the characteristic myositis related antibodies which react with amino acid t-RNA synthetases.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41070 02 ARB
(formerly
Z01 AM 41070-01 ARB)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The nature of the DNA anti-DNA antibodies in sera of patients with SLE

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Paul H.. Plotz	Senior Investigator	ARB/NIAMS
	Mary Cronin	Medical Staff Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH
ARB NIAMS

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DNA will be isolated from the immune complexes of patients with lupus and the sequences determined after cloning in plasmids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41071 02 ARB
(formerly
Z01 AM 41071-01 ARB)

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A controlled trial of apheresis in treatment of poly/dermatomyositis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Paul H. Plotz	Senior Investigator	ARB/NIAMS
	Mary Cronin	Medical Staff Fellow	ARB/NIAMS
	Frederick W. Miller	Medical Staff Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*

Foreign: None

Blood Bank, CC, NIH Dept. Radiology, CC, NIH; MDB/NIDDK
Dept. of Rehabilitation, CC, NIH; Lab of Oral Medicine, NIDR, NIH

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Section on Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS/NIH/ Bethesda, MD 20892

TOTAL MAN-YEARS: 2	PROFESSIONAL: 2	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Severe polymyositis or dermatomyositis which responds poorly to corticosteroids may be difficult to treat. Because of anecdotal reports suggesting efficacy, we are comparing plasmapheresis and leukapheresis with a sham procedure. Patients who fail to respond will be offered pulse intravenous cyclophosphamide

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41072-02 ARB
(formerly
Z01 AM 41072-01 ARB)

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cyclosporin A in Rheumatoid Arthritis (RA)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.H. Klippel, Senior Investigator, ARB/NIAMS
R.L. Wilder, Senior Investigator, ARB/NIAMS
D. Yocum, Medical Staff Fellow, ARB/NIAMS
L. Gerber, Chief, Rehab/CC
J. Minor, Pharmacy/CC
C. Yarboro, Nursing/CC
C. Berkebile, Nursing/CC

COOPERATING UNITS *(if any)*

Rehabilitation/CC, Pharmacy/CC, Nursing/CC   Foreign: None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2	PROFESSIONAL: 2	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The efficacy and toxicity of daily, oral cyclosporin A (CSA) in patients with active rheumatoid arthritis is being evaluated in a 6 month double-blind study of low dose (1mg/kg/day) versus high dose (10mg/kg/day) CSA. Toxicity is monitored weekly for the first 2 months and then every 2 weeks until 6 months. Disease activity is assessed on a bi-monthly basis. Patients who do not develop at least a 40% improvement in disease activity at 6 months are dropped from the study, while those with a 40% or greater response continue for an additional 6 months of drug therapy. The effects of CSA on various measures of T lymphocyte function in peripheral blood and within the synovium is studied in all patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AR 41073-01 ARB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
High dose methotrexate with leucovorin rescue in rheumatoid arthritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: J.H. Klippel, Senior Investigator, ARB/NIAMS
J.B. Shiroky, LOM/NIDR
B.A. Chabner, DCT/NCI
C.J. Allegra, DCT/NCI
J. Minor, Pharmacy/CC

COOPERATING UNITS *(if any)*
DCT/NCI, Pharmacy/CC Foreign: None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIAMS/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This pilot study is evaluating high dose intravenous methotrexate (500mg/meter square) with leucovorin rescue in the management of patients with severe, active rheumatoid arthritis. Patients must have failed at least one second line agent or high dose corticosteroid therapy to be entered. Methotrexate and leucovorin is given every 2 weeks for a total of 4 doses. Patients are evaluated during this period and for an additional 4 months for changes in arthritis activity and complications related to therapy. The effects of high-dose methotrexate on various measures of lymphocyte function and cell populations in the peripheral blood is studied.

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